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Studies on chemosensitivity of the megascolecid earthworm *Lampito mauritii*

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With 2 figures

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1. Introduction

Soil *p*H has been reported to be highly correlated with earthworm numbers and laboratory experiments have demonstrated the sensitivity of certain species of earthworms to hydrogen ion concentration (SATCHELL 1955). The sensitivity of earthworms to chemical stimulation is a subject of clear ecological significance having implications for the choice of habitat which they make (ARRHENIUS 1921, BODENHEIMER 1935, SATCHELL 1955, KHALAF EL-DUWEINI & GHABBOUR 1964). Sense organs which react to chemical stimuli are on the prostomium or the buccal epithelium which comes into contact with substances when the buccal chamber is everted. The reactions of earthworms to salts, acids, bases and the distribution in soil in relation to *p*H have also been documented by GATES (1978).

Sensitivity to *p*H and chemical stimulation in earthworms has been demonstrated by several workers using a variety of methods under different experimental conditions (HURWITZ 1910, SATCHELL 1955, JOHNSON 1971). Presently five important biological buffers with different and overlapping *p*H values made of different chemical components and concentrations have been employed to study chemosensitivity as a behavioural response in *Lampito mauritii*.

2. Material and methods

2.1. Experimental animals

Individuals of *Lampito mauritii* (15 ± 2 cm in length and 0.8 ± 0.1 g in mass) collected from sandy loam soils of Madras were maintained in the dark in native soil with about 20 g% moisture. Experiments were conducted between 21 h and 24 h under red illumination of a photographic bulb (640 nm) to which earthworms are known to be insensitive.

2.2. Experimental set up

The apparatus consists of a round, graduated, open ended glass tube, 31 cm long and 1 cm diameter, with a side arm of similar diameter, at its midpoint, and only 2.5 cm long (Fig. 1). Earthworms were allowed to crawl through the tube and as they approached the midpoint a drop of buffer was added through the side arm with the help of a fine needled syringe. The apparatus was cleaned after every trial.

2.3. Experimental solutions

Standard stock solutions were prepared of Walpole's M/100 acetate-acetic acid buffer (after LILLIE 1965; *cf.* GABE 1976), Walpole's M/5 acetate-acetic acid buffer (*cf.* HUMASON 1972), Gomori's M/5 phosphate buffer (*cf.* HUMASON 1972), SØRENSEN's M/15 phosphate buffer (*cf.* HUMASON 1972) and HOLMES's boric acid-borax buffer (*cf.* HUMASON 1972). *p*H of the solutions were tested in a L1-120 Elico *p*H meter. All reagents used were of the finest grade.

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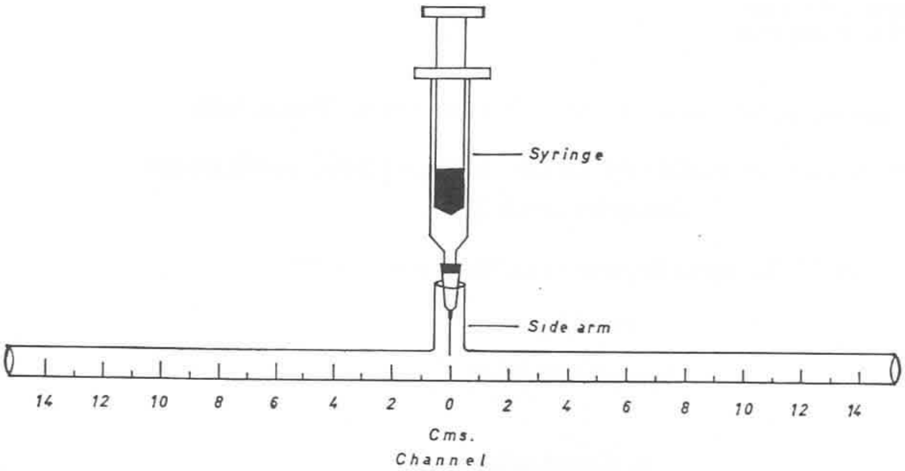

Fig. 1. Apparatus used to study withdrawal response.

2.4. Experimental design

The hypodermic syringe (described in 2.2.) filled with the test solution was held in such a way that a drop of test solution hung suspended from the tip of the needle. As the worm approached near the tip of the needle the drop was allowed to fall on the prostomium and the first one or two segments of the worm. The worm then produced a quick withdrawal response which typically consisted of an initial rapid contraction of the anterior portion of the body followed by arrest of the head and extension of the posterior body; a second withdrawal occurred subsequently. In the present study only the initial anterior contraction was recorded, the point at which the prostomium first comes to rest being read from the graduation marked on the side of the glass channel. Withdrawal from the needle point was scored as a positive sign and progress without withdrawal as negative. In the event of the drop from the needle falling too quickly or too late or either missing the worm entirely or hitting segments posterior to the prostomium, the worm was allowed to leave the channel and was discarded.

3. Results and observations

Results of the present experiments are given in Table 1 and Fig. 2 for M/100 acetate-acetic acid buffer (M/100 AAB), M/5 acetate-acetic acid buffer (M/5 AAB), M/5 phosphate buffer (M/5 PB), M/15 phosphate buffer (M/15 PB) and boric acid-borax buffer (BBB).

As the worms showed violent avoidance reaction at *p*H 2.70 to 3.98 of M/5 AAB, withdrawal response could not be measured. Worms showed no response at *p*H 5.29 and *p*H 5.59 of M/15 PB. The maximum responses recorded for M/100 AAB, M/5 AAB, M/5 PB, M/15 PB and BBB were 3.28 cm (*p*H 3.37), 4.22 cm (*p*H 4.04), 4.56 cm (*p*H 7.70), 3.28 cm (*p*H 8.04) and 2.78 cm (*p*H 9.0) respectively; and the minimum were 0.08 cm (*p*H 6.77), 0.50 cm (*p*H 6.5), 0.8 cm (*p*H 5.9), 0.24 cm (*p*H 5.91) and 0.06 cm (*p*H 7.4) respectively.

A decline in withdrawal response from *p*H 3.37 to 6.77 of M/100 AAB, from *p*H 4.04 to 6.5 of M/5 AAB, and an increased response from *p*H 5.9 to 7.7 of M/5 PB, from *p*H 5.29 to 8.04 of M/15 PB and from *p*H 7.4 to 9.0 of BBB is clearly evident (Table 1, Fig. 2). Effect of the component solutions of different buffers is also provided in Table 2. A comparison of the withdrawal responses of the worms to different *p*H in all the five buffers can also be read from Table 1 and Fig. 2.

4. Discussion

When earthworms are exposed to *p*H solutions the prostomium is simultaneously subjected to chemical stimulation of varying concentrations as well as to the *p*H of the solution. The present experiment in many respects reveals interesting information about the nature of chemosensitivity in earthworms.

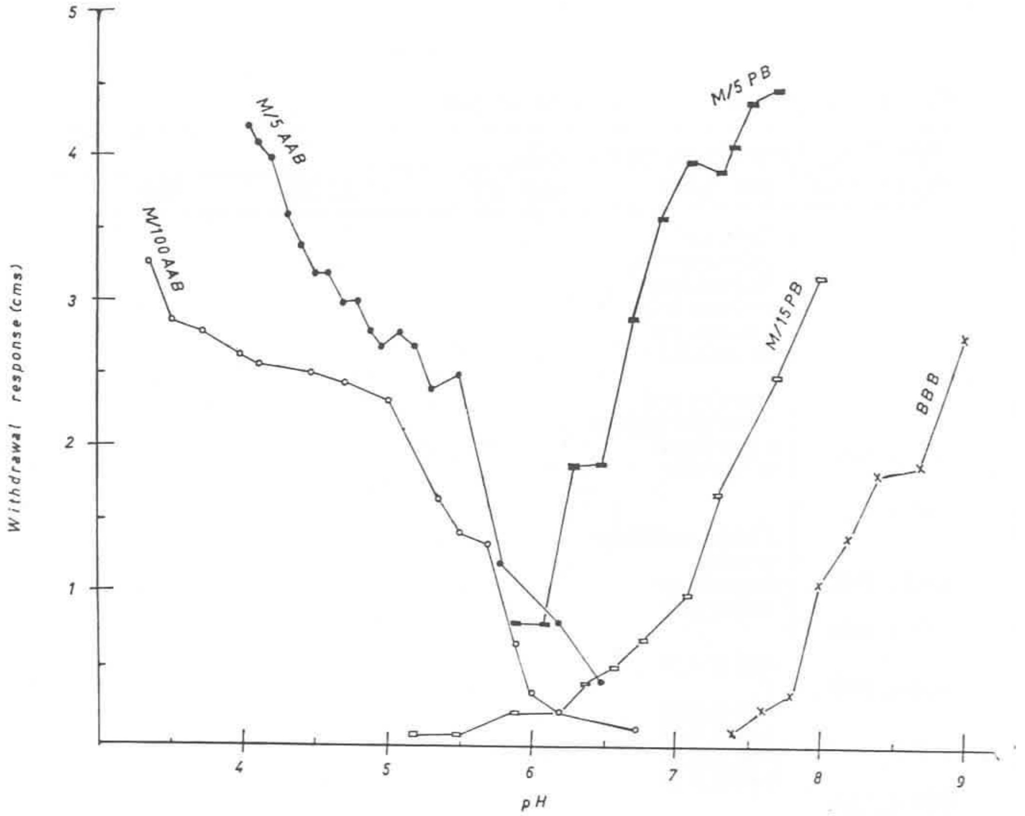

Fig. 2. Withdrawal response of *L. mauritii* to *p*H of different buffers.

The pattern of avoidance behaviour between *p*H 2.7 and 3.9 of M/5 AAB and the gradual decrease in the positive response from 4.22 cm to 0.5 cm as the *p*H is increased from 4.04 to 6.5 again confirms that earthworms are capable of differentiating between neutrality and increasing acidity; i.e., hydrogen ion concentration may influence withdrawal response of the earthworms to M/5 AAB of varying *p*H. To confirm, the experiment was repeated with M/100 AAB whose molarity was one-twentieth of M/5 AAB. Though the withdrawal response of the worms showed a gradual decline as the *p*H was increased from 3.37 to 6.77 (Table 1, Fig. 2) the withdrawal response was comparatively lower than that observed for M/5 AAB at every *p*H. This makes evident that the withdrawal response of the worms is influenced more by the molarity of the buffer component than by the *p*H of the buffer as such. When the prostomial region of the worms were exposed directly to M/100 and M/5 sodium acetate and M/100 and M/5 acetic acid solutions which form the respective components of M/100 AAB and M/5 AAB, worms showed negligible withdrawal response to M/100 and M/5 sodium acetate solution whereas a very pronounced response was noticed to M/100 and M/5 acetic acid (Table 2). It can be thus considered that the withdrawal response of the worms to M/100 AAB and M/5 AAB may probably be mainly due to the concentration of acetic acid and not sodium acetate. In other words the monobasic sodium salt has not interfered with the withdrawal response of the earthworms.

Withdrawal response of *L mauritii*, to *p*H in the alkali range, when exposed to BBB offering a *p*H range 7.4 to 9.0 shows a gradually increasing response from 0.06 cm at *p*H 7.4 to 2.78 cm at *p*H 9.0 (Table 1, Fig. 2). On exposure to the buffer components of the BBB, there was no response to M/5 boric acid solution while a response of 3.66 cm was recorded

Table 1. Withdrawal response of *L. mauritii* to various buffers1)

*p*H	Withdrawal response in cm, mean ± S.D.				
	M/100 AAB	M/5 AAB	M/5 PB	M/15 PB	BBB
2.70		Twisting,, convulsive jerking and exuding coelomic fluid, response not measurable			
2.80					
2.91					
3.08					
3.14		Twisting and convulsive jerking response not measurable			
3.20					
3.31					
3.37	3.28 ± 0.69				
3.41					
3.52	2.88 ± 0.61	Violent avoidance reaction response not measurable			
3.59					
3.72					
3.73	2.82 ± 0.55				
3.90					
3.98	2.64 ± 0.48				
4.04		4.22 ± 0.38			
4.11	2.58 ± 0.53				
4.16		4.10 ± 0.20			
4.27		4.02 ± 0.17			
4.36		3.66 ± 0.67			
4.45		3.46 ± 0.59			
4.47	2.52 ± 0.50				
4.53		3.28 ± 0.69			
4.62		3.20 ± 0.62			
4.71	2.46 ± 0.49	3.08 ± 0.55			
4.80		3.04 ± 0.53			
4.90		2.86 ± 0.65			
4.99		2.74 ± 0.61			
5.00	2.34 ± 0.45				
5.11		2.80 ± 0.57			
5.22		2.64 ± 0.53			
5.29				No response	
5.37	1.66 ± 0.31				
5.38		2.40 ± 0.32			
5.50	1.42 ± 0.34				
5.57		2.54 ± 0.40			
5.59				No response	
5.71	1.34 ± 0.34				
5.89		1.26 ± 0.73			
5.90			0.80 ± 0.67		
5.91				0.24 ± 0.24	
6.00	0.32				
6.10			0.88 ± 0.61		
6.21		0.88 ± 0.43			
6.22	0.20				
6.24				0.26 ± 0.25	
6.30			1.92 ± 0.34		
6.47				0.40 ± 0.20	
6.50		0.50 ± 0.38	1.92 ± 0.34		
6.64				0.50 ± 0.43	
6.70			2.92 ± 0.40		
6.77	0.08				
6.81				0.78 ± 0.66	
6.90			3.68 ± 0.85		
6.98				0.92 ± 0.68	
7.10			4.00 ± 0.28		
7.17				1.00 ± 0.54	
7.30			3.96 ± 0.40		

Table 1. (Continued)

pH	Withdrawal response in cm, mean ± S.D.				
	M/100 AAB	M/5 AAB	M/5 PB	M/15 PB	BBB
7.38				1.70 ± 0.92	
7.40			4.10 ± 0.35		0.06
7.50			4.48 ± 0.42		
7.60					0.20
7.70			4.56 ± 0.60		
7.73				2.50 ± 0.86	
7.80					0.30
8.00					1.06 ± 0.76
8.04				3.28 ± 1.03	
8.20					1.38 ± 0.85
8.40					1.82 ± 0.92
8.70					1.88 ± 0.46
9.00					2.78 ± 0.96

1) Value per pH per buffer is mean of 25 individual observations.

Table 2. Effects of buffer components on the withdrawal response of the earthworm *L. mauritii*2)

Buffer	Component	Withdrawal response in cm, mean ± S.D.
M/100 acetate-acetic acid buffer	a) M/100 acetic acid	3.28 ± 0.69
	b) M/100 sodium acetate	0.08
M/5 acetate-acetic buffer	a) M/5 acetic acid	Violent avoidance reaction Not measurable
	b) M/5 sodium acetate	0.50 ± 0.38
M/5 phosphate buffer	a) M/5 dibasic sodium phosphate	Violent avoidance reaction Not measurable
	b) M/5 monobasic sodium phosphate	No response
M/15 phosphate buffer	a) M/15 dibasic sodium phosphate	3.56 ± 0.72
	b) M/15 potassium acid phosphate	No response
Boric acid-borax buffer	a) M/20 borax (sodium borate)	3.66 ± 0.67
	b) M/5 boric acid	No response

2) Value per pH per buffer is mean of 25 individual observation.

for M/20 borax solution (Table 2). The increased withdrawal response to increased pH may be either due to the concentration of hydroxyl ions in the buffer or due to the chemical nature of the buffer component, M/20 borax (sodium borate).

The experiments were repeated under similar experimental design with phosphate buffers of different molarities that gave pH grades which overlap with those of M/100 AAB and M/5 AAB. This experimental phase is of great significance on the following grounds:

(a) It facilitates comparison of withdrawal response to different buffers.

(b) Observation of withdrawal response at identical pH and identical response at different pH would bring to light more information about the chemosensitivity of earthworms.

M/5 PB offered a pH range 5.9 to 7.7 whereas M/15 PB offered 5.29 to 8.04. One of the components common to both the phosphate buffers, but in different molarities, is dibasic sodium phosphate. As the phosphate buffers overlap the acetate-acetic acid buffers in a certain range of pH, a comparative study of the withdrawal responses of earthworms was made possible. It is of significance to note that the withdrawal response of the worms differed markedly in spite of the fact that the pH was maintained the same by the different buffers (Fig. 2). At pH 5.9 and 6.1 the response in M/5 AAB > /5 PB > /100 AAB > /15 PB. However, the response was more to M/5 PB than to the rest between pH 6.2 and 7.7. As already mentioned withdrawal response of the worms showed a sharp decline as the pH was increased from 4.04 to 6.5 in the case of M/5 AAB and from 3.37 to 6.77 in the case of M/100 AAB, the converse of which is observed when exposed to the two phosphate buffers where the worms show a sharp increase in their withdrawal response as pH tends towards 7.0. This can be explained only on the basis of sensitivity of earthworms to chemical agents. Dibasic sodium phosphate is a strong alkali and earthworms are more sensitive to the concentration of this chemical than to the hydrogen ion concentration of the phosphate buffers. Positive response of the worms even at the neutral pH 7 supports this view.

With a view to further confirm the effect of chemostimulation by the components of the buffer systems a supplementary experiment was designed. This consisted of exposing the prostomial region of the worms to each of the components individually. An extremely low or zero response was observed towards M/5 sodium acetate, M/100 sodium acetate, M/5 monobasic sodium phosphate and M/15 potassium acid phosphate solutions whose salts are of monobasic nature. Similarly no response was observed towards M/5 boric acid solution (Table 2). On the contrary withdrawal response was high towards the complementary solutions viz M/5 acetic acid, M/100 acetic acid, M/5 dibasic sodium phosphate, M/15 dibasic sodium phosphate and M/20 borax (sodium borate). These results clearly demonstrate that in phosphate buffers, it is the dibasic sodium phosphate that evokes high withdrawal response and not the corresponding monobasic component. Similarly in the case of BBB, withdrawal response may be due to the dibasic sodium salt rather than pH.

The increased withdrawal response of the worms to M/5 AAB and M/5 PB compared to M/100 AAB and M/15 PB respectively is an indication of the molar concentration of the components deciding the intensity of the withdrawal response rather than the pH. This means that earthworms can not only detect chemical substances but also their concentrations, which throws light on the findings of MANGOLD (1953) who reported that acids such as phosphoric, tartaric, citric, oxalic and malic found in plant materials are accepted at low concentrations but not at higher ones. The present work also demonstrates that earthworms are not only sensitive to certain chemicals but also to their concentrations.

There is still much to be investigated about the earthworms' response to stimulation by chemical substances and pH. It can however be presently inferred that withdrawal response of earthworms depends more on the chemical properties and concentrations of chemicals rather than pH of the solution as such.

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Synopsis: *Original scientific paper*

MAGDOOM, K. M. M., & S. A. ISMAIL, 1986, Studies on chemosensitivity of the megascolecid earthworm *Lampito mauritii*. Pedobiologia **29**, 229—235.

The behavioural response of the earthworm *Lampito mauritii* to different biological buffers is observed.

Higher withdrawal responses to M/5 acetate-acetic acid buffer compared to M/100 acetate-acetic acid buffer, and for M/5 phosphate buffer compared to M/15 phosphate buffer suggest that the molarities of buffer components have a direct influence on the withdrawal response.

Withdrawal responses by the earthworm to different buffers at identical pH suggest that contraction response varies more according to chemical properties and concentrations of the components than to the pH of the buffers as such.

Key words: Oligochaeta, Megascolecidae, Lampito mauritii, chemosensitivity, behavioural responses, pH, buffer.